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Pharmacokinetics and tissue distribution of novel traditional Chinese medicine-platinum anticancer agents in rats

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Abstract

The pharmacokinetics and tissue distribution profiles of a novel series of traditional Chinese medicine-platinum (TCM-Pt) compounds $[Pt(C_8H_8O_5)(NH_2R)_2]$: **1** (where R = H), **3** (R = CH_3) and **5** (R = C_6H_{10}), were studied in Sprague–Dawley rats following a single bolus intravenous (i.v.) injection. Platinum concentrations in total plasma, plasma ultrafiltrate, urine and tissues were measured by flameless atomic absorption spectroscopy. Pharmacokinetic studies showed that plasma concentrations of total and free platinum for the novel TCM-Pt compounds as well as cisplatin and carboplatin declined in a biexponential manner with a short distribution half-life ($t_{1/2\alpha}$: 0.12–0.34 h). Compared with cisplatin, the novel TCM-Pt compounds had a longer elimination half-life ($t_{1/2\beta}$), larger dose normalized area under the curve (AUC/D), larger volume of distribution at steady-state (V_{ss}), slower clearance (CL) of free platinum and higher percentage of cumulative urinary excretion (CUE), which can be attributed to their lower chemical reactivities. In tissues, the highest Pt concentrations were found in the kidney, followed by the liver and the lowest in the heart; no Pt was detected in the brain. Twenty-four hours after drug administration, platinum concentrations in tissues were significantly lower for the novel TCM-Pt compounds. These findings suggest that the novel compounds might afford higher clinical efficacy and reduced systemic side effects, when compared with cisplatin.

Keywords: TCM-Pt compounds; Cisplatin; Carboplatin; Protein binding; Pharmacokinetics; Tissue distribution

1. Introduction

Cisplatin (Fig. 1) is a widely used and highly effective antitumor agent for the treatment of many cancers including breast, lung, head and neck, ovary and testis [1]. However, it is markedly toxic to many normal tissues, and in particular nephrotoxicity has been a major problem [2,3]. More significantly, acquired resistance to cisplatin has limited the drug's clinical applications [1].

In order to reduce the adverse effects of cisplatin, much effort has been made to develop platinum (Pt)-based antitumor drugs with lower toxicity. It has been found that modification of cisplatin to contain less labile leaving groups alters the toxicity of the drug. Carboplatin (Fig. 1), a second-generation Pt drug with a cyclobutanedicarboxylate ligand replacing the chloride leaving groups, demonstrated good antitumor activity and decreased nephrotoxicity [4,5]. The development of carboplatin was predominantly focused on improving patients' quality of life during Pt-based chemotherapy, however it also suffers cross-resistance to cisplatin [6]. Oxaliplatin, a diaminocyclohexane (DACH)-containing third-generation Pt-based anticancer drug is the first of this class to be approved for the treatment of colorectal cancer [7] (Fig. 1). It has a spectrum of activity and mechanisms of action and resistance that is different from cisplatin and carboplatin [8,9].

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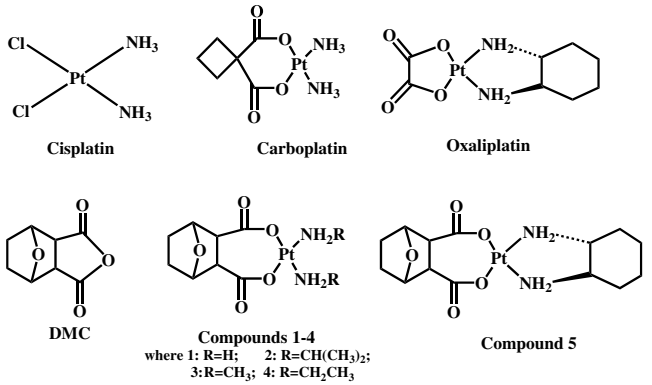

Fig. 1. Chemical structures of cisplatin, carboplatin, oxaliplatin, demethylcantharidin (DMC) and novel TCM-Pt compounds.

We recently reported a novel series of Pt anticancer agents $[Pt(C_8H_8O_5)(NH_2R)_2]$ (**1**–**5**) that integrated a Pt-moiety with demethylcantharidin (DMC), a modified structural component of a traditional Chinese medicine (TCM) (Fig. 1) [10]. Cantharidin is the naturally occurring active principle of *Epicanta gorhami* or *Mylabris* (''blister beetles''), which has been widely used as a TCM for the treatment of lung, liver and digestive tract tumors, but it has severe side effects such as dysphagia, hematemesis, and dysuria [11]. DMC is a synthetic analogue of cantharidin that is less toxic and has antitumor activity. The idea for combining the DMC ligand with a Pt-moiety was based on an ''East meets West'' philosophy in the drug design strategy. The intention was to exploit the benefits of TCM and to utilize the well-established chemical and biological properties of a conventional western antiumor agent such as cisplatin, in creating new chemical entities with specific therapeutic use. Indeed, the integration of the DMC ligand with different Pt-moieties rendered the novel TCM-Pt compounds to have *in vitro* and *in vivo* antiproliferative activity that were superior to cisplatin or carboplatin and with lower toxicity [10,12]. The novel complexes were also shown to have the ability to circumvent cisplatin resistance and to exhibit protein phosphatase 2A (PP2A) inhibitory activity [13]. In light of the promising *in vitro* and *in vivo* biological evaluations, these compounds have potential to be further developed as new Pt-based anticancer drugs; and thus there is merit in characterizing the pharmacokinetics of the novel compounds in animals.

In this study, the pharmacokinetics and tissue distribution profiles of the novel TCM-Pt compounds were examined in rats. Parallel studies were conducted with cisplatin and carboplatin, mainly to determine whether the comparative pharmacokinetic and distribution patterns of these drugs would provide an explanation for the lower toxicity of the novel complexes. TCM-Pt compounds **1**, **3** and **5** were chosen for the tissue distribution studies because **1** is analogous to cisplatin; **3** has high water solubility; and **5** demonstrated excellent antitumor activity.

2. Experimental

2.1. Chemicals and reagents

TCM-Pt complexes $[Pt(C_8H_8O_5)(NH_2R)_2]$: **1** (where R = H), **3** (R = CH_3) and **5** (R = C_6H_{10}) were synthesized by reacting demethylcantharidin (prepared from a Diels–Alder reaction between furan and maleic anhydride, followed by Pd–C catalyzed hydrogenation), with a series of $(NH_2R)_2Pt(NO_3)_2$ (prepared from treatment of K_2PtCl_4 with potassium iodide and appropriate primary amines, followed by reaction with silver nitrate) [10]. After washing with ice water, ethanol and diethyl ether in sequence, and drying at 65 °C, the complexes were characterized by infrared, ^{1}H NMR (500 MHz, D_2O, $SiMe_4$): complex **1**: δ 4.92 (m, 2H), 4.02 (m, 2H), 1.72 (m, 4H); complex **3**: δ 4.85 (m, 2H), 3.86 (m, 2H), 2.21 (s, 6H), 1.65 (m, 4H); complex **5**: δ 4.85 (m, 2H), 4.00 (m, 2H), 2.38 (m, 2H), 2.06 (m, 2H), 1.76 (m, 4H), 1.59 (m, 2H), 1.31 (m, 2H), 1.18 (m, 2H); and elemental analysis (CHN): calcd for complex **1** $C_8H_{14}N_2O_5Pt$ (%): C, 23.25; H, 3.41; N, 6.27; found: C, 23.45; H, 3.48; N, 6.70; calcd for complex **3** $C_{10}H_{18}N_2O_5 \cdot H_2O$ (%): C, 26.14; H, 4.36; N, 6.10; found: C, 26.26; H, 4.00; N, 6.11; calcd for complex **5** $C_{14}H_{22}O_5N_2Pt \cdot 2H_2O$ (%): C, 31.75; H, 4.91; N, 5.29; found: C, 32.09, H. 4.48; N, 5.50.

Cisplatin and carboplatin were obtained from Strem (Newburyport, MA, USA). All other chemicals were of the best grade available.

2.2. Preparation of doses

Cisplatin was dissolved in normal saline (NS), whereas carboplatin and compounds **1**, **3** and **5** were dissolved in 5% dextrose in water immediately before use. Cisplatin, compound **3** and carboplatin were given at a single dose of 7.5, 78 and 98 mg/kg respectively, which corresponded to their i.v. 10% lethal dose (LD_{10}). Compounds **1** and **5** were given at a single dose of 8 mg/kg, as limited by their solubility.

2.3. Animals

Experimental animals were obtained from and bred at the Laboratory Animal Service Center (LASEC) at the Chinese University of Hong Kong. Male Sprague–Dawley rats (90–210 g) were housed in an air-conditioned environment (temperature: 23 ± 2 °C; relative humidity: 55 ± 5%) and kept on a light/dark cycle of 12 h/12 h. The animals had free access to standard rat chow and water. All experiments involving animals were approved by the Chinese University of Hong Kong Animal Ethics Committee and were carried out under the supervision of the University LASEC.

One day prior to pharmacokinetic studies, each animal was prepared with two polyethylene cannulas (ID 0.40 mm, OD 0.80 mm, Portex Ltd., Hythe, Kent, Eng-

land) inserted into the right jugular vein under light anesthesia (diethyl ether). A surgical incision was made on the ventral side of the neck to expose the jugular vein, which was cannulated with two polyethylene tubes that were led under the skin and exteriorized at the back of the neck. The exposed areas were surgically sutured and the rats were placed into individual metabolic cages designed to collect urine separately from feces. The rats were allowed to recover and were fasted overnight with free access to water.

2.4. Analytical methodology

Platinum concentrations were analyzed by flameless atomic absorption spectroscopy (FAAS) (sample: 20 μL; wavelength: 265.9 nm; apparatus: Perkin–Elmer Analyst 100 atomic absorption spectrometer with graphite cell atomizer HGA800) [14,15]. Pt standard solution for FAAS (solvent: 0.2% HNO_3) was used to construct the calibration curve, which was linear for the concentration range of 50–500 ppb of Pt. At Pt concentrations >500 ppb, samples were appropriately diluted with 0.2% HNO_3. The coefficient of variance for the assay was less than 4% at $\geqslant$5 ppb.

2.5. Plasma protein binding

An ultrafiltration method was employed to determine the plasma protein binding of the five drugs [16,17]. Rat plasma was freshly prepared prior to experiment by centrifugation at 3000*g* for 5 min. Stock solutions of cisplatin, carboplatin, compounds **1**, **3** and **5** in water were freshly prepared (1 mg/mL). Aliquots of the platinum drug solutions were added to the plasma to obtain four final concentrations of 5, 20, 50, 120 μg/mL for each drug. After incubation at 37 °C for 24 h, the sample was immediately ultrafiltrated with a 10 K MW cutoff filter membrane (UFC 3GC membrane, Millipore, Tokyo, Japan) at 12,000*g* at 4 °C for 30 min. The ultrafiltrate was digested by 65% HNO_3 at 70 °C overnight, then diluted by 0.2% nitric acid. Control experiments were carried out in water instead of plasma.

The binding kinetic studies were carried out at a drug concentration of 20 μg/mL. At time points of 5, 15, 30 min, and at 1, 1.5, 2, 4, 8 and 24 h, 200 μL of the samples were taken and Pt concentrations were analyzed by FAAS as described above. Each experiment was repeated three times.

Protein binding was calculated by the following equation:

$$\%\ \text{protein binding} = (1 - C_u/C) \times 100$$

where C_u is the concentration of Pt from the ultrafiltrate of plasma and C is that of the control.

2.6. Pharmacokinetics

Rats were randomly divided into five groups of five animals in each. Drugs were given by i.v. injection from one of the cannulas. Heparinized normal saline (20 IU/mL, 0.2 mL) was used to flush the cannula immediately after dosing. Blood (0.5 mL) was sampled through the other i.v. cannula prior to drug administration and at 5, 10, 15, 30 min, as well as 1, 1.5, 2, 4, 6, 8, 12, and 24 h after drug injection. After blood sampling, an equal volume of heparinized normal saline solution (20 IU/mL) was injected back into the rat via the cannula for flushing and to prevent blood coagulation. Blood samples were placed in heparinized eppendorfs and plasma was immediately separated by centrifugation at 3000*g* for 5 min. Aliquots of plasma were frozen at −80 °C until analysis to determine total platinum concentrations. A separate aliquot of plasma was ultrafiltrated with a 10 K MW cutoff filter membrane (UFC 3GC membrane, Millipore, Tokyo, Japan) at 12,000*g* at 4 °C for 30 min. The ultrafiltrates were similarly kept at −80 °C until analysis for Pt. Urine was collected over a period of 24 h and the volume measured; the samples were kept at −80 °C until analysis.

Plasma samples (0.1 mL) were digested by 65% nitric acid (0.9 mL), followed by heating at 70 °C overnight, and dilution by 0.2% nitric acid. Urine samples were diluted directly by 0.2% nitric acid. Pt concentrations were analyzed by FAAS.

The concentrations of total and ultrafiltrable platinum were best characterized by WinNonLin softwear (version 2.1, Pharsight Corporation, Mountain View, NC, USA) using an i.v. two-compartment model.

2.7. Tissue distribution

Rats were randomly divided into groups of five animals. Drugs were given by iv injection from the cannula. At times 10 and 30 min, then 1, 4, 8, 12 and 24 h after drug administration, the animals were sacrificed by cutting the inferior vena cava. The liver, kidney, lung, stomach, intestine, brain, spleen and heart were excised, washed in normal saline and blotted dry with filter paper. An accurately weighed amount of tissue (~0.5 g) was added to 65% nitric acid [15]. The sample was refluxed at 140 °C for 6 h, then diluted to an appropriate concentration and analyzed by FAAS.

2.8. Statistical analysis

Statistical analyses were performed with student *t*-test. *P*-Values <0.05 were considered significantly different. All data were expressed as mean ± SD.

3. Results

3.1. Plasma protein binding

When compound **1** was incubated with plasma, the percentage of free platinum fell rapidly to less than 20% in 24 h, and the extent of protein binding was independent of concentration at 5, 20, 50 and 120 μg/mL (Table 1).

Table 1
In vitro protein binding studies of platinum complexes incubated in rat plasma at 37 °C for 24 h

Compounds	Concentration (μg/mL)	$t_{1/2}$ of free Pt (h)	% of protein-bound Pt at 24 h
Compound **1**	5		84.83 ± 0.33
	20	4.02 ± 0.23	88.12 ± 0.18
	50		81.90 ± 0.52
	120		87.70 ± 0.82
Compound **3**	5		77.96 ± 0.48
	20	5.18 ± 0.25	83.45 ± 0.28
	50		84.92 ± 0.26
	120		80.66 ± 2.69
Compound **5**	5		91.34 ± 0.17
	20	1.09 ± 0.04	91.65 ± 0.21
	50		92.37 ± 0.21
	120		90.78 ± 0.85
Cisplatin	5		95.23 ± 0.22
	20	1.33 ± 0.02	96.50 ± 0.16
	50		95.75 ± 0.13
	120		96.00 ± 0.03
Carboplatin	5		65.79 ± 0.56
	20	10.38 ± 1.76	53.71 ± 1.17
	50		48.20 ± 0.73
	120		40.11 ± 3.13

Values are mean ± SD of three experiments.

The half-life for the disappearance of free Pt of **1,** determined at an initial concentration of 20 μg/mL was 4.02 h. The extent of protein binding of **3** and **5** were also independent of concentration. Compound **5** bound to plasma protein very rapidly as indicated by a half-life of 1.09 h, and after 24 h incubation, more than 90% was protein bound. Protein binding of compound **3** ($t_{1/2} = 5.18$ h) was slower than **1** and **5**, and after 24 h incubation, about 80% was protein bound.

The protein-binding behavior of all three novel TCM-Pt compounds was more akin to cisplatin than carboplatin. Cisplatin bound very rapidly to plasma protein with a half-life of 1.33 h, and more than 95% of the drug was protein bound after 24 h. However, carboplatin bound to plasma protein very slowly ($t_{1/2} = 10.38$ h) and to a much lesser extent. As the concentration of carboplatin increased, the percent of protein-bound Pt decreased significantly, as shown in Table 1.

3.2. Pharmacokinetics of platinum drugs in plasma

After drug administration, measurable platinum include ultrafiltrable platinum, consisting of non-protein-bound intact drug and metabolites; and total platinum, representing all platinum species, both protein-bound or -unbound. The concentration versus time curves generated for total and free platinum in plasma obtained from rats treated with compounds **1**, **3**, **5**, cisplatin and carboplatin are shown in Fig. 2 and the pharmacokinetic parameters are summarized in Tables 2 and 3.

After an i.v. injection of 8 mg/kg of **1**, a peak plasma level for total Pt of 11.68 μg/mL was reached immediately and decreased to 0.54 μg/mL within 2 h. Twenty-four hours after administration, the total Pt in plasma decreased to 0.13 μg/mL. Plasma levels of free Pt decreased more rapidly; to 0.08 μg/mL within 2 h and was undetectable after 2 h (Fig. 2c). A rapid distribution phase ($t_{1/2\alpha}$) and a slow elimination phase ($t_{1/2\beta}$) were noted for compound **1**. The rapid decay of free Pt led to a much shorter elimination half-life $t_{1/2\beta}$ (0.33 ± 0.07 h) when compared with that of the total Pt (19.18 ± 5.11 h) (Tables 2 and 3). Consequently, this resulted in a smaller $AUC_{0-\infty}$ for free Pt (4.19 ± 0.21 μg/mL h) than for total Pt (14.20 ± 2.24 μg/mL h). Similarly, plasma levels of free Pt decreased more

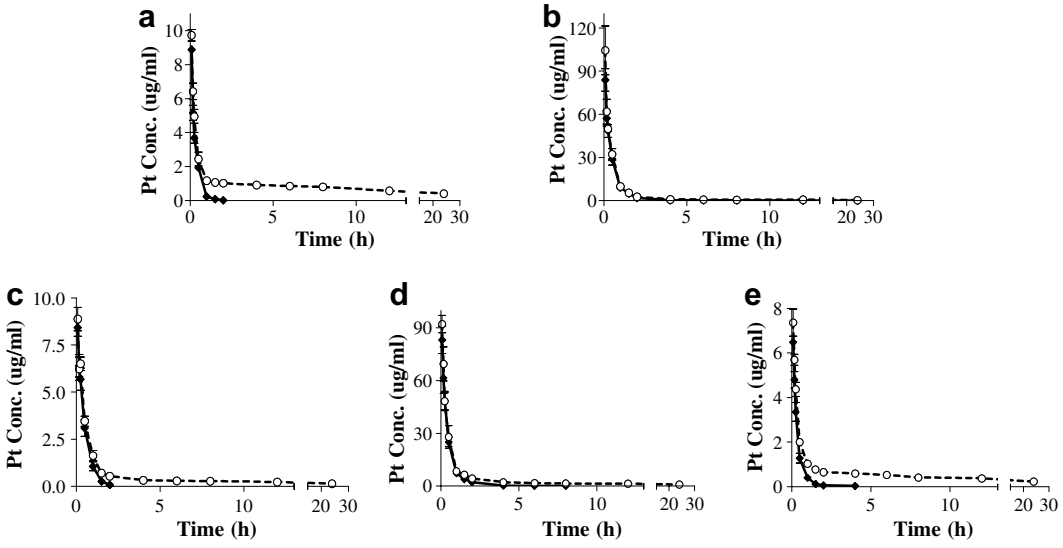

Fig. 2. Concentration–time curves generated for total (dashed line) and free Pt (solid line) in the plasma of Sprague–Dawley rats given a single bolus i.v. injection of: (a) cisplatin (7.5 mg/kg); (b) carboplatin (98 mg/kg); (c) compound **1** (8 mg/kg); (d) compound **3** (78 mg/kg) and (e) compound **5** (8 mg/kg). Each point represents the mean ± SD of five independent experiments.

Table 2
Pharmacokinetic parameters of total platinum after i.v. administration of compounds **1** (8 mg/kg), **3** (78 mg/kg), **5** (8 mg/kg), cisplatin (7.5 mg/kg) and carboplatin (98 mg/kg) in Sprague–Dawley rats

Compound	Carboplatin	**3**	**1**	**5**	Cisplatin
$t_{1/2\alpha}$ (h)	$0.32 \pm 0.02^{*}$	$0.28 \pm 0.03^{*}$	$0.34 \pm 0.03^{*}$	$0.20 \pm 0.01^{\#}$	$0.16 \pm 0.03^{\#}$
$t_{1/2\beta}$ (h)	$10.78 \pm 2.02^{*}$	$16.32 \pm 6.10^{\#}$	19.18 ± 5.11	$14.58 \pm 1.03^{\#}$	$16.06 \pm 3.92^{\#}$
$AUC_{0-\infty}$ (μg/mL h)	57.55 ± 4.40	88.35 ± 12.42	14.20 ± 2.24	16.09 ± 0.86	27.35 ± 4.72
$AUC_{0-\infty}/D$ (kg/h/L)	$1.12 \pm 0.09^{*}$	$2.56 \pm 0.36^{*,\#}$	$3.76 \pm 0.59^{*,\#}$	$5.35 \pm 0.27^{\#}$	$5.61 \pm 0.99^{\#}$
V_{ss} (L/kg)	$4.28 \pm 0.60^{*}$	5.35 ± 1.55	$4.99 \pm 0.87^{*}$	3.37 ± 0.16	$3.70 \pm 0.24^{\#}$
CL (L/h/kg)	$0.89 \pm 0.07^{*}$	$0.40 \pm 0.06^{*,\#}$	$0.26 \pm 0.06^{*,\#}$	$0.19 \pm 0.01^{\#}$	$0.18 \pm 0.03^{\#}$
24 h cumulative urinary excretion (CUE)%	$88.10 \pm 13.80^{*}$	$93.72 \pm 16.92^{*}$	$78.77 \pm 8.53^{*}$	$63.46 \pm 4.62^{\#}$	$57.22 \pm 8.91^{\#}$

Values are mean ± SD of five replicates.
$t_{1/2\alpha}$: distribution half-life; $t_{1/2\beta}$: elimination half-life; $AUC_{0-\infty}$: area under the plasma concentration–time curve; $AUC_{0-\infty}/D$: dose-normalized AUC; V_{ss}: volume of distribution at steady-state; CL: total clearance.
* $P < 0.05$ compared with cisplatin.
# $P < 0.05$ compared with carboplatin.

Table 3
Pharmacokinetic parameters of free platinum after i.v. administration of compounds **1** (8 mg/kg), **3** (78 mg/kg), **5** (8 mg/kg), cisplatin (7.5 mg/kg) and carboplatin (98 mg/kg) in Sprague–Dawley rats

Compound	Carboplatin	**3**	**1**	**5**	Cisplatin
$t_{1/2\alpha}$ (h)	$0.34 \pm 0.01^{*}$	$0.30 \pm 0.04^{*}$	$0.21 \pm 0.08^{\#}$	$0.19 \pm 0.01^{*,\#}$	$0.12 \pm 0.05^{\#}$
$t_{1/2\beta}$ (h)	$3.34 \pm 0.36^{*}$	$1.78 \pm 0.80^{*,\#}$	$0.33 \pm 0.07^{\#}$	$2.05 \pm 0.57^{*,\#}$	$0.33 \pm 0.07^{\#}$
$AUC_{0-\infty}$ (μg/mL h)	40.30 ± 1.28	40.21 ± 2.76	4.19 ± 0.21	2.60 ± 0.10	2.97 ± 0.17
$AUC_{0-\infty}/D$ (kg/h/L)	$0.78 \pm 0.06^{*}$	$1.17 \pm 0.08^{*,\#}$	$1.10 \pm 0.05^{*,\#}$	$0.82 \pm 0.03^{*}$	$0.61 \pm 0.03^{\#}$
V_{ss} (L/kg)	$0.92 \pm 0.06^{*}$	$0.51 \pm 0.04^{*,\#}$	$0.36 \pm 0.02^{*,\#}$	$0.80 \pm 0.07^{*,\#}$	$0.47 \pm 0.05^{\#}$
CL (L/h/kg)	$1.26 \pm 0.04^{*}$	$0.86 \pm 0.06^{*,\#}$	$0.90 \pm 0.05^{*,\#}$	$1.22 \pm 0.05^{*}$	$1.65 \pm 2.68^{\#}$

Values are mean ± SD of five replicates.
$t_{1/2\alpha}$: distribution half-life; $t_{1/2\beta}$: elimination half-life; $AUC_{0-\infty}$: area under the plasma concentration–time curve; $AUC_{0-\infty}/D$: dose-normalized AUC; V_{ss}: volume of distribution at steady-state; CL: total clearance.
* $P < 0.05$ compared with cisplatin.
# $P < 0.05$ compared with carboplatin.

rapidly than that of total Pt, after i.v. administration of compound **5** or cisplatin (Fig. 2a and e).

Compared to **1**, free Pt after i.v. administration of compound **3** decreased much more slowly (Fig. 2d). The total and free Pt curves for **3** were very similar initially (1 h) and after 2 h, the ratio of free-to-total Pt was ~0.50, whilst that for compound **1** was ~0.14, thus concurring with the results for protein binding. A much shorter elimination half-life of the free Pt (1.78 ± 0.80 h) was observed than for the total Pt (16.32 ± 6.10 h) (Tables 2 and 3). The clearance of free Pt was about twice as fast as that of total Pt, whereas the $AUC_{0-\infty}$ for the total Pt was twice that of free Pt.

For carboplatin, both total and free Pt were detectable after 24 h (Fig. 2b). Pt was mostly recovered in the ultrafiltrate, particularly in the first 2 h following drug administration, indicating that most of carboplatin in the plasma was present as non-protein-bound Pt. The elimination half-life of total Pt was 10.78 ± 2.02 h, which was longer than that of the free Pt (3.34 ± 0.56 h). $AUC_{0-\infty}$ for total Pt was 57.55 ± 4.40 μg/mL h, which was slightly higher than that of free Pt (40.30 ± 1.28 μg/mL h), and clearance of total Pt was slower than that of free Pt.

From Tables 2 and 3, it was found that for all five drugs, the volume of distribution at steady-state (V_{ss}) were very large, indicating that these drugs may be widely distributed in tissue. The V_{ss} of free Pt for carboplatin was the largest (0.92 ± 0.06 L/kg), followed by **5** (0.80 ± 0.07 L/kg) and **3** (0.51 ± 0.04 L/kg). For cisplatin, the V_{ss} was 0.47 ± 0.05 L/kg and **1** was 0.36 ± 0.02 L/kg, which were considerably smaller than that of carboplatin. The protein binding ability of carboplatin was found to be the lowest, thus it is reasonable that the V_{ss} of carboplatin was the largest amongst the five drugs.

The clearance (CL) of total Pt for cisplatin was very slow (0.18 ± 0.03 L/h/kg), in contrast to the rapid clearance of carboplatin (0.89 ± 0.07 L/h/kg). The CL of compounds **1**, **3** and **5** were faster than cisplatin, but slower than carboplatin. However, the CL of free Pt for cisplatin was the fastest amongst the five drugs, most likely as a result of protein binding.

The percentage of Pt excreted in the urine over a 24 h period after drug administration (CUE) is also an important parameter. The CUE was highest for carboplatin ($88.10 \pm 13.80\%$) and **3** ($93.72 \pm 16.92\%$), followed by **1** ($78.77 \pm 8.53\%$), **5** ($63.46 \pm 4.62\%$) and lowest for cisplatin ($57.22 \pm 8.91\%$) (Table 2).

3.3. Tissue distribution

After an intravenous bolus administration of cisplatin, Pt distributed rapidly and peak Pt levels were reached within 10 min for all tissues investigated (Fig. 3). The highest Pt concentrations were found in the kidney, followed by

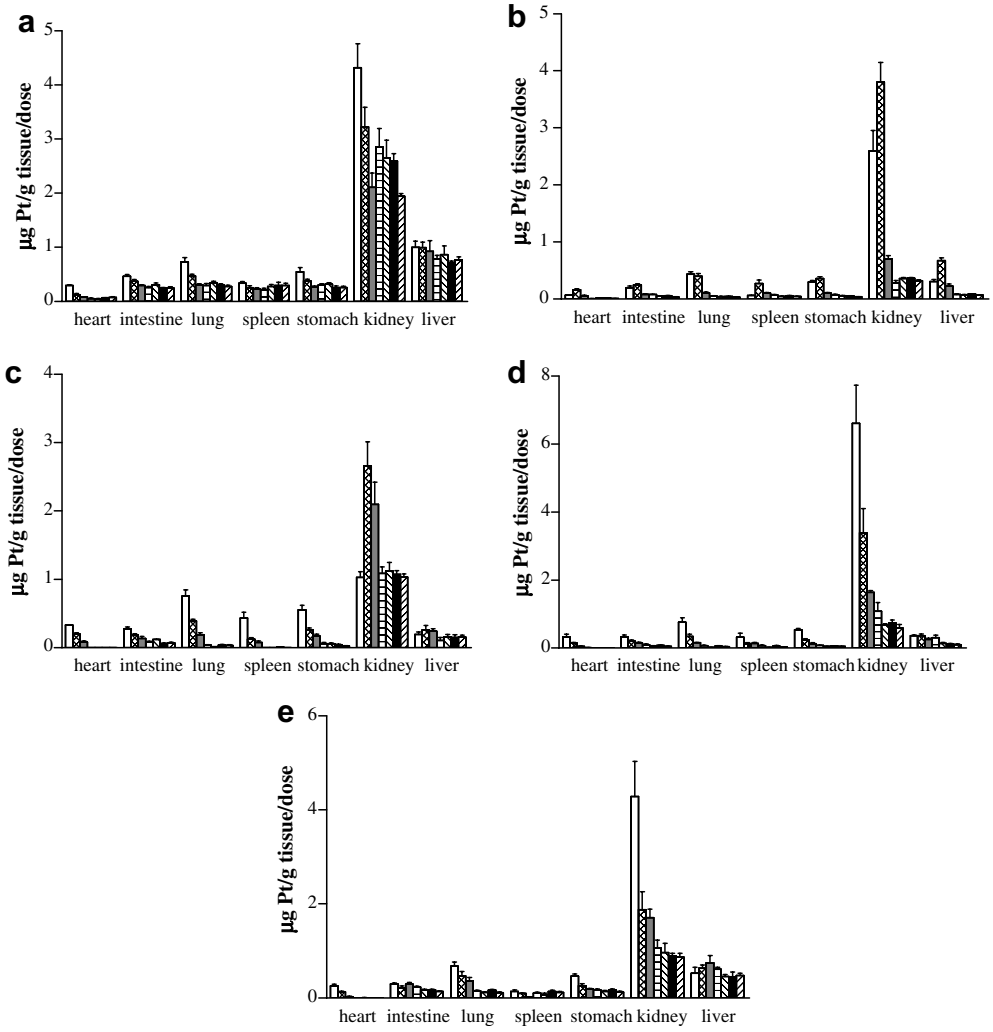

Fig. 3. Concentrations of Pt in various tissues at times 0.17, 0.5, 1, 4, 8, 12 and 14 h after an i.v. administration of: (a) cisplatin (7.5 mg/kg); (b) carboplatin (98 mg/kg); (c) compound **1** (8 mg/kg); (d) compound **3** (78 mg/kg) and (e) compound **5** (8 mg/kg). Each point represents the mean ± SD of five independent experiments.

the liver. Ten minutes after cisplatin treatment, Pt concentration in the kidney (2.81 ± 0.20 μg Pt/g tissue/dose) was four times higher than that in the liver (0.65 ± 0.56 μg Pt/g tissue/dose), and at later time points, Pt concentrations in the kidney were consistently three times higher than that in the liver. The lowest Pt concentrations were found in the heart (0.19 ± 0.01 μg Pt/g tissue/dose at 10 min) and spleen (0.22 ± 0.02 μg Pt/g tissue/dose at 10 min). The removal of Pt appeared to be biphasic: most were removed at between 10 min and 1 h, and the remaining concentration decreased slowly with time. The faster elimination phase may be due to normal biological eliminations such as urinary excretion; and the slow reduction of Pt level at later times may be a result of irreversible protein binding.

Similar to cisplatin, Pt concentration in the kidney was much higher than in other tissues after carboplatin treatment (Fig. 3). Peak Pt concentrations were found at 0.5 h for all the tissues, which suggested that carboplatin distributed more slowly than cisplatin. This concurred with the longer distribution half-life ($t_{1/2\alpha}$) observed for carboplatin.

The tissue distribution profiles for compounds **1**, **3** and **5** also showed the highest Pt concentration in the kidney, and peak Pt concentrations for most tissues were reached within 10 min (Fig. 3). The majority of Pt was removed after 4 h and the remaining Pt decreased slowly with time.

The Pt concentrations of the five platinum drugs in different tissues 24 h after i.v. administration were compared (Table 4). For all drugs tested, Pt was not detected in brain tissues, likely due to their inability to cross the blood brain barrier, which concurs with previous reports of the slow uptake of cisplatin into the brain [18]. But for the other seven tissue types, the highest Pt levels were found for cisplatin and longer retention was noted. This result suggests that the effusion of Pt from those organs occurred more slowly for cisplatin than for the other compounds. For example, 24 h after drug administration, the Pt concentration in the kidneys for **1** (1.03 ± 0.05 μg Pt/g tissue/dose), **3** (0.59 ± 0.11 μg Pt/g tissue/dose) and **5** (0.87 ± 0.08 μg Pt/g tissue/dose) were all significantly lower than that for cisplatin (1.94 ± 0.05 μg Pt/g tissue/dose). Amongst the novel

Table 4
Platinum concentrations expressed as (μg Pt/g tissue)/dose (mg/kg) in tissues 24 h after given a single bolus iv injection of compounds **1** (8 mg/kg), **3** (78 mg/kg), **5** (8 mg/kg), cisplatin (7.5 mg/kg) and carboplatin (98 mg/kg) in Sprague–Dawley rats

Tissue	Compound **1**	Compound **3**	Compound **5**	Carboplatin	Cisplatin
Heart	ND	$0.003 \pm 0.001^{*,\#}$	ND	$0.01 \pm 0.001^{*}$	$0.07 \pm 0.02^{\#}$
Intestine	$0.07 \pm 0.01^{*,\#}$	$0.05 \pm 0.02^{*}$	$0.14 \pm 0.01^{*,\#}$	$0.03 \pm 0.01^{*}$	$0.25 \pm 0.03^{\#}$
Lung	$0.04 \pm 0.01^{*}$	$0.04 \pm 0.01^{*}$	$0.11 \pm 0.01^{*,\#}$	$0.03 \pm 0.001^{*}$	$0.28 \pm 0.02^{\#}$
Spleen	ND	$0.028 \pm 0.007^{*,\#}$	$0.12 \pm 0.02^{*,\#}$	$0.04 \pm 0.004^{*}$	$0.29 \pm 0.04^{\#}$
Stomach	$0.02 \pm 0.005^{*,\#}$	$0.05 \pm 0.006^{*,\#}$	$0.12 \pm 0.01^{*,\#}$	$0.04 \pm 0.003^{*}$	$0.26 \pm 0.02^{\#}$
Kidney	$1.03 \pm 0.05^{*,\#}$	$0.59 \pm 0.11^{*,\#}$	$0.87 \pm 0.08^{*,\#}$	$0.32 \pm 0.02^{*}$	$1.94 \pm 0.05^{\#}$
Liver	$0.16 \pm 0.02^{*,\#}$	$0.10 \pm 0.02^{*,\#}$	$0.48 \pm 0.05^{*,\#}$	$0.06 \pm 0.003^{*}$	$0.77 \pm 0.06^{\#}$

Values are means ± SD ($n = 5$).
ND: not detectable.
* $p < 0.05$, compared with cisplatin.
$^{\#}$ $p < 0.05$, compared with carboplatin.

TCM-Pt compounds, the highest Pt accumulation was found for **5**. The Pt concentrations in the intestine, lung, spleen, stomach and liver after administration of **5** were all much higher than that for **1** and **3**. This is in agreement with the pharmacokinetic results that free Pt of **5** had a much larger volume of distribution ($V_{ss} = 0.80 \pm 0.07$ L/kg) than **1** ($V_{ss} = 0.36 \pm 0.02$ L/kg) and **3** ($V_{ss} = 0.51 \pm 0.04$ L/kg).

4. Discussion

The *in vitro* antitumor activity of the novel TCM-Pt complexes **1**, **3** and **5** (IC_{50}s in a human hepatocellular carcinoma cell line SK-Hep1 were 20.59, 15.44 and 2.97 μM respectively), were previously found to be superior to cisplatin (IC_{50} of 54.15 μM) and carboplatin (IC_{50} of 500.56 μM) [10]; and the *in vivo* antitumor effect of the novel TCM-Pt complexes in a liver xenograft model have also been established [12]. However, the pharmacokinetics and tissue distribution of the compounds have not been determined. In contrast, the pharmacokinetics of cisplatin and carboplatin is well documented [19,20], and it has been reported that cisplatin binds to a large number of proteins, primarily to albumin (~50%), β-globulins and immunoglobulins [21]. The major binding site apparently involves a Met-S, *N*-macrochelate, and other minor monofunctional sites featuring Met-S and Cys-34 [22]. Thus the main factor that influences protein binding is likely to be the reactivity of the Pt compounds. This concurs with our recent report on the reactivity trend of the novel TCM-Pt compounds and cisplatin and carboplatin towards S-containing amino acids and nucleophiles, where it was found to be in the order of: cisplatin > 5 > 1 > 3 > carboplatin [23].

In this current study, we report for the first time, the pharmacokinetics and biodistribution of novel compounds **1**, **3** and **5** in rat plasma and tissues, and compared with that of the reference drugs cisplatin and carboplatin. Ultrafiltrable platinum (free Pt) is responsible for the antitumor activity and toxicity of platinum drugs; Pt irreversibly bound to plasma proteins is considered to be pharmacologically inactive [24]. Thus the description of the pharmacokinetics of platinum drugs should include both the platinum in total plasma (total Pt) and that in plasma ultrafiltrate (free Pt).

It was found that for all five drugs, both the total and free platinum had very short distribution half-lives ($t_{1/2\alpha}$), which suggested rapid distribution from blood plasma into tissues (Tables 2 and 3). The distribution half-lives of total and free Pt were similar, thus the decrease in total Pt concentration is likely to be associated with the fate of free Pt. The shorter distribution half-lives of free Pt for cisplatin, compounds **1** and **5** indicated that these drugs may distribute more rapidly into tissues than carboplatin, which was confirmed by peak Pt concentrations occurring 10 min post-administration in most tissues.

The elimination half-lives ($t_{1/2\beta}$) of free Pt for the five drugs were in the order of: carboplatin > **5** > **3** > **1** ≈ cisplatin. Cisplatin bound to plasma protein quickly, which led to the rapid disappearance of free Pt. Carboplatin was the most stable, binding slowly to protein and had a longer elimination half-life that was ten times longer than cisplatin (Table 3). The elimination half-life for **5** was much longer than cisplatin and compound **1**; a characteristic that is similar to its diaminocyclohexane (DACH) analogue, oxaliplatin [24]. Unlike free platinum, the elimination half-life for total platinum of carboplatin was the shortest, but the $t_{1/2\beta}$ for the five drugs was not significantly different (Table 2).

The area under the curve (AUC) is considered an important determinant of the therapeutic effect of Pt-based drugs. For example, it was found that during the treatment of patients with germ cell tumors of the testis, no relapses occurred in patients whose AUCs were greater than 4 mg/ml min, while relapse occurred in five out of eight patients who had an AUC of under 4 mg/ml min [25]. In this study, the drugs were administered at different doses, thus it is more reasonable to compare the dose-normalized $AUC_{0-\infty}$, which is expressed as $AUC_{0-\infty}/D$. As free platinum is considered to be the pharmacologically active species, it is more important to compare the $AUC_{0-\infty}/D$ of the free platinum drugs (Table 3). From the results, no obvious trend was observed, but the $AUC_{0-\infty}/D$ of cisplatin (0.61 ± 0.03 kg/h/L) was the lowest, likely to be caused by its high reactivity towards plasma protein. Significantly, it was found that the novel TCM-Pt

compounds had relatively higher $AUC_{0-\infty}/D$: compound **5** (0.82 ± 0.03 kg/h/L) was comparable to carboplatin (0.78 ± 0.06 kg/h/L); and that of **1** (1.10 ± 0.05 kg/h/L) and **3** (1.17 ± 0.08 kg/h/L) were higher than carboplatin. This implies that for the novel compounds there will be more free drugs in the body, and thus a higher therapeutic effect may be attainable.

It is noteworthy that the CUEs of the three novel TCM-Pt compounds were all higher than cisplatin, particularly that of compound **3**. This can once again, be explained by their different reactivities, leading to different protein binding characteristics. It has been reported that the lower urinary excretion of cisplatin might be due to a tubular reabsorptive process [26]. The higher degree of proximal tubular platination would increase the intracellular platinum concentration, which may explain the high nephrotoxicity of cisplatin [27]. A high percentage of protein binding, being irreversible, will also lead to a low urinary excretion of cisplatin. Unlike cisplatin, the urinary excretion of carboplatin is by glomerular filtration [28]. Irreversible protein binding in the plasma is much slower with carboplatin than with cisplatin, together with slower rates of binding to the kidney and other tissues. These aspects can contribute to a higher percentage of urinary excretion of carboplatin. The novel TCM-Pt compounds were less protein bound and showed higher CUEs than cisplatin; thus it is reasonable to expect these compounds to be less nephrotoxic due to a decreased accumulation of platinum in the kidney. Lower nephrotoxicity of the novel compounds was indeed demonstrated in a recent study using a porcine kidney LLC-PK-1 cell line model [23].

The volume of distribution (V_{ss}) is influenced by the lipophilic character of the drug, as well as by protein binding. The incorporation of the DACH ligand into compound **5** renders it more lipophilic and is likely to have facilitated the compound's rapid passage across cell membranes, thus resulting in a large V_{ss}. Oxaliplatin, with a similar DACH moiety, also has a large volume of distribution, which is believed to contribute to the drug's lack of nephrotoxicity [24].

Although there were quantitative differences, the tissue distribution profiles were similar for the five drugs. In the eight tissues examined, the highest Pt concentration was found in the kidney for all compounds. This is in agreement with previous studies of cisplatin and carboplatin [29,30]. The brush border of the proximal tubule contains an abundance of three sulfhydryl groups [31]. The total number of protein-bound thiol groups in the kidney is strongly reduced after cisplatin administration, especially in the mitochondrial fraction and this reduction in –SH groups is attributed to direct binding by Pt [27]. The disproportionate accumulation of cisplatin within the kidney compared with other organs, and its retention for days after a single dose, may be the most critical aspect of its nephrotoxicity [32]. The lower reactivity of the novel TCM-Pt compounds and carboplatin to –SH may explain their lower Pt accumulation in the kidney.

Platinum levels detected in the liver were second to the kidney after the administration of the five drugs. Although relatively high amounts of Pt were found in the liver, a previous study by this research group found that none of the novel compounds caused undue hepatotoxicity [12]. One explanation might be the large storage capacity of the liver and its efficient detoxification by substances such as metallothioneins that are produced in large quantities [33]. Alternatively, it might be that most hepatocytes are resting in the G_0 phase, during which the cytotoxicity of Pt drugs is lower [18].

In summary, the pharmacokinetics and tissue distribution profiles can explain the lower toxicity of the novel TCM-Pt compounds, which in turn, is correlated to their chemical reactivities. The novel TCM-Pt compounds are less reactive than cisplatin and thus exhibited reduced protein binding, larger AUCs, higher CUEs, and less tissue accumulation. Since the concentration of the novel TCM-Pt compounds decreased quickly and lower Pt accumulations were found in the tissues and plasma, these characteristics suggest the compounds have potential for reducing systemic side effects in cancer chemotherapy and a higher dose of the drug might be attainable, in order to optimize the antitumor efficacy. Combined with their significant *in vitro* and *in vivo* antitumor activities, these novel TCM-Pt compounds are promising candidates for clinical evaluation.

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